Primer

The double life of the prion protein

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The increasingly high-profile prion protein is involved in a class of devastating neurological diseases, including human Creutzfeld–Jakob disease, scrapie in sheep and goats, and 'mad cow disease'. It is highly conserved among mammalian species, is found on the surface of cells that produce it, and is expressed in many tissues, including the brain. Like the superhero Batman, the prion protein may lead a double life. Just as one must get to know Batman's alter ego — mild-mannered Gotham City millionaire Bruce Wayne — to understand the caped crusader, understanding the role of the prion protein in disease may require an appreciation of the function of its seemingly innocuous cellular form.

An infectious protein?

Scrapie is a neurodegenerative disease of sheep which is characterized by spongiform degeneration of the brain and the accumulation of a fibrillar protein aggregate in the brain. (Mad cow disease, or bovine spongiform encephalopathy (BSE) is the analogous disease in cattle.) Carleton Gadjusek demonstrated more than thirty years ago, in work for which he was awarded the 1976 Nobel Prize, that scrapie could be transmitted from one animal to another *via* intracerebral injection of a homogenate of infected brain tissue. Gadjusek called the infectious agent a 'slow-virus', but unlike any known virus, no specific nucleic acid component of the infectious agent has been detected. In response to this paradox, J.S. Griffith postulated in 1967 that the agent contained only protein (the 'protein-only' hypothesis), and suggested several possible mechanisms by which such an entity could self-replicate.

In the early 1980s, a single protein was identified as the major component both of the scrapie-associated fibrils in diseased brain and of brain preparations enriched in scrapie infectivity. Surprisingly, the amino-acid sequence of this disease-associated protein seems to be identical to that of a normal cellular protein. Also in the early 1980s, Stanley Prusiner coined the name 'prion' (protein infectious virion) to reflect his belief that the disease-associated protein — designated the prion protein scrapie-associated form, or PrP^{Sc} — was the one to which Griffith's hypothesis referred. The normal cellular form of the protein is designated PrP^{C}.

The idea that a disease-associated protein can in some way recruit a normal cellular protein to become disease-causing is contrary to much of conventional thinking about disease-causing agents; the idea has yet to be universally accepted, not least because a number of aspects of the transmission of related spongiform encephalopathies remain to be understood (see box). For example, it seems that both BSE and scrapie can be transmitted to other

How are prion protein diseases transmitted?

The pressing public health issues concerning prions involve disease transmission, especially the possibility that BSE is transmitted from cattle to humans; there is some evidence that eating BSE-infected beef might be responsible for the emergence of a new strain of Creutzfeldt–Jakob disease in humans. Some critical questions about prion transmission, and speculations on the answers, are as follows.

How are prion diseases transmitted from one individual to another?
It is not at all clear how, for example, scrapie is passed from one sheep to another. There are examples of spongiform encephalopathies that seem to be passed on by eating infectious tissue – for example, BSE in cattle is thought to result from their being fed infectious material, and kuru, a human disease, is thought to be passed on by cannibalistic ritual. In addition, there is some evidence that scrapie can be passed on to animals by others in their herd or group, and perhaps even by contact with the same food or bedding material as infected animals.

How did the original PrP^{Sc} arise, and is it possible to contract a prion disease without being infected or inheriting a mutant form of PrP?
It is possible that there is a very low spontaneous rate of conversion from the normal cellular form to the 'scrapie' form of the protein. If the disease-associated form is capable of self-replication (see text and Fig. 1), a small amount of 'seed' would be necessary for conversion of PrP^{C} to PrP^{Sc}. And if the disease-associated form is infectious, once it has replicated in one individual it might be able to pass to another. In addition, very rare mutations might convert the normal prion protein to a disease-associated form that aggregates or is transmitted more readily.

Can mutations in the *PrP* gene cause inherited diseases?
There are inherited spongiform encephalopathies that may be attributable to mutant forms of the PrP protein. How this causes disease is not yet clear.

If the infectious agent is an ordered polymer of PrP^{Sc}, how can it get into the brain, across the blood–brain barrier?
This remains a mystery, but several lines of evidence suggest that the lymph nodes provide a way-station *en route* to the brain. Perhaps the agent is taken up by nerve terminals and travels retrogradely up to the central nervous system.

How can we test the likelihood of bovine–human transmission?
Studies with laboratory animals, such as mice and hamsters, have already provided a lot of information on the behaviour of PrP. Perhaps similar studies using rodents as well as primates will, together with increasing amounts of epidemiological evidence, provide new insights.

Can the PrP^{C}–PrP^{Sc} conversion be inhibited?
A number of strategies based on perturbing the presumed folding intermediates are being actively investigated.

animals, such as mice or hamsters, but the transmitted disease retains some characteristics of either BSE or scrapie in the recipient animal. How is this possible if both diseases rely on the same single cellular protein for their perpetuation? Even 'strain-specific' characteristics of a single disease, such as how long it takes to develop and precisely which parts of the brain it affects, are transmitted with the infectious agent. Again, it is hard to see how a single protein can achieve this.

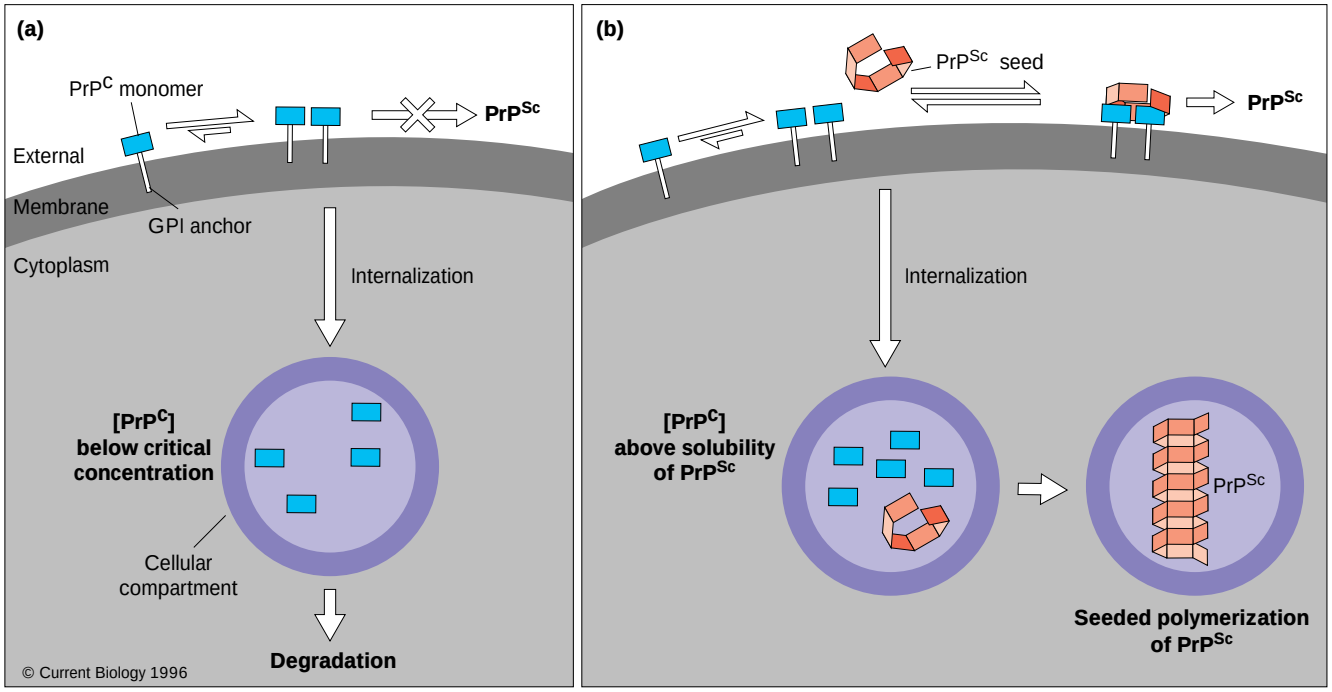
In this article, we will consider a model for the behavior of the normal and disease-associated prion proteins, and the extent to which this model explains what we know about transmissible spongiform encephalopathies. We will refer to the infectious entity as 'the agent' rather than the prion, in order to avoid confusing it with PrP^{Sc}, as the protein-only hypothesis has yet to be proven definitively, for example by a demonstration that PrP^{Sc} produced *in vitro* is infectious.

Infection by a protein-only agent

The normal, cellular form of the prion protein is not infectious. It is expressed in many tissues and by many cell types but has no known function. Mice lacking the *PrP* gene, first produced by Charles Weissmann and colleagues, are viable, but have altered neuronal function and develop neurological abnormalities later in life. Strikingly, these '*PrP* knockout' mice are resistant to infection by the scrapie agent.

The failure to detect covalent chemical differences between the scrapie-associated PrP^{Sc} and the normal PrP^{C} led James Hope to suggest that they might be conformational isomers of one another, an idea originally postulated by Griffith and recently supported by spectroscopic studies. According to the protein-only hypothesis, infectious PrP^{Sc} in an innoculum must be able to convert host PrP^{C} into additional copies of PrP^{Sc}, thus replicating itself. We and our colleagues have demonstrated that this conversion can take place outside the cell, in a system containing only PrP^{Sc} isolated from infected brain and PrP^{C} produced in cell culture. The conversion *in vitro* is most effective when the PrP^{C} and PrP^{Sc} used in the

Figure 1



A proposal to explain the involvement of PrP^{C} and PrP^{Sc} in the replication of PrP^{Sc}. The tendency of the prion protein to aggregate may be important in its normal funciton (**a**), as well as its disease-associated behavior (**b**). (a) The normal function of PrP^{C}, which is anchored to the cell surface by a glycosyl-phosphatidyl inositol (GPI) tail, is unclear, but might involve controlled two-dimensional oligomerization in the plane of the membrane. The functional form of PrP^{C} may be a monomer, but could also be a dimer (as shown), or a higher oligomer. The oligomeric forms could be stabilized and prevented from proceeding to a PrP^{Sc} nucleus by attachment to the membrane and/or specific interactions with another cell-surface molecule. Nucleation of PrP^{Sc} would not occur in a cellular compartment either, provided that the PrP^{C} concentration was kept below the 'critical concentration'. (b) In scrapie, PrP^{Sc} replication could be initiated by an interaction between an extracellular PrP^{Sc} seed with any form of cell-surface PrP^{C} (the dimer is shown). This interaction could inhibit the normal function of PrP^{C} and seed its ordered polymerization, producing more PrP^{Sc} at the cell surface (provided that the inhibitory factors postulated in (a) could be circumvented), or, after internalization, in a cellular compartment (provided that the concentration of PrP^{C} in the compartment exceeded the thermodynamic solubility of PrP^{Sc}). Accumulation of PrP^{Sc} could block normal degradative and/or recycling pathways and lead to progressive neuronal dysfunction.

assay are derived from the same species, and it can propagate the biochemical properties associated with specific disease strains. These results suggest that other endogenous factors are not required for the conversion to occur, although such factors may normally be involved *in vivo*.

The conversion of PrP^{C} to PrP^{Sc} *in vitro* could take place by means of nucleated polymerization, with PrP^{Sc} acting as a seed which induces polymerization of PrP^{C}, or *via* the formation of a heterodimer containing one monomer of Prp^{Sc} and one of PrP^{C}. Mechanistic studies of the conversion *in vitro* strongly support the nucleated polymerization model; we believe that PrP^{Sc} is a polymeric seed which replicates itself by inducing PrP^{C} to polymerize *in vivo* and produce additional PrP^{Sc}, as outlined in Figure 1. For the purposes of the discussion below, we define a polymer to be a heterogeneous collection of multimers, each capable of growth. In contrast, an oligomer is a defined multimeric species which is not capable of further polymerization.

Neurodegeneration

Mice lacking the *PrP* gene can develop progressive coordination problems (as do scrapie-infected sheep and BSE-infected cattle). Thus, PrP^{Sc}-induced neurodegeneration may be partly due to loss of normal PrP^{C} function (the effect might be indirect, for example, if PrP^{C} affects neural development in a manner which becomes evident only in aged animals). Neurodegeneration in infectious scrapie is clearly more rapid than that observed in the *PrP* knockout mice, however, arguing that PrP^{Sc} itself is neurotoxic.

Adriano Aguzzi and Charles Weissmann have examined the roles of PrP^{Sc} and PrP^{C} in neuronal degeneration by grafting brain tissue from transgenic mice which overexpress PrP^{C} (5–8-fold higher levels than in wild-type mice) into the brain of an otherwise identical mouse lacking the *PrP* gene. After inoculation with PrP^{Sc}, the graft underwent rapid spongiform degeneration but no degeneration of the adjacent tissue lacking PrP^{C} was observed, despite the fact that this tissue contained an amount of PrP^{Sc} easily sufficient to transmit the disease. This result suggests that both cell-surface PrP^{C} and extracellular PrP^{Sc} are required for neurodegeneration to occur; PrP^{Sc} may be a pathogenic ligand for PrP^{C} (see Fig. 1). This model does not explain why degeneration is apparently specific to the brain even though PrP^{C} expression is widespread. The answer to this question may involve the role played by PrP^{C} in the unique biology of the neuron.

Function of the cellular prion protein

The function of the cellular prion protein PrP^{C}, which is anchored to the cell-surface by a glycolipid attachment, is unknown. Its sequence is highly conserved, which usually implies that a protein has some important function. But the mild phenotype observed in some *PrP* knockout mice — reduced long-term potentiation of synaptic transmission, which is normally associated with learning, and sleep pattern abnormalities — suggests that whatever the normal role of PrP^{C}, it can be at least partially fulfilled by other proteins.

Point mutations in the *PrP* gene can lead to inherited human neurological diseases which resemble transmissible scrapie, such as Creutzfeldt–Jakob disease, Gerstmann–Straussler–Scheinker disease and fatal familial insomnia. It is difficult to understand why a gene sequence that is only a single mutation away from protein aggregation and neurological disaster should be conserved. We propose a solution to this paradox. The normal function of PrP^{C} may require its controlled oligomerization under conditions which prevent the uncontrolled polymerization that would lead to PrP^{Sc} formation. The oligomerized normal protein could be involved in cell–cell interactions, synapse formation or membrane protein recycling. The elucidation of this normal function could hold the key to understanding the pathogenesis of scrapie and related spongiform encephalopathies.

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